Comparison of two different techniques for deepithelialization: a split-mouth case series

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Abstract

Aim: The aim of the present preliminary study was to observe and make a histologic comparison of connective tissue grafts (CTGs) harvested from the lateral palatal mucosa through the use of two different harvesting techniques.

Materials and methods: Three patients were enrolled in the study, providing six standardized CTGs. One well-experienced periodontist collected the replacement grafts using two different methods. After outlining the grafts to a fixed dimension, the graft on one side was deepithelialized by a round coarse bur intraorally before harvesting. The graft on the contralateral side was obtained by harvesting from the palate first; subsequently, deepithelialization was performed extraorally with the aid of a no. 15c blade. After finalization, histologic evaluation was performed.

Results: No apparent differences were found between the two observed techniques in terms of graft thickness, proportion, and composition. After deepithelialization, epithelial remnants were clearly evident in five out of six cases. Despite being more technique sensitive, the removal of epithelium by bur scored better. Proper graft handling and graft regularity are described as advantages of the more conventional epithelial excision by blade.

Conclusions: Despite the wide use and broad variety of commonly applied techniques of graft deepithelialization, the present authors assume that full excisions with the use of a blade are hardly ever achieved. Despite the unpredictable retrieval of epithelium by blade, graft handling and graft regularity can be proposed as the biggest advantages. On the other hand, the presented novel in situ deepithelialization with a round bur seems to be more predictable.

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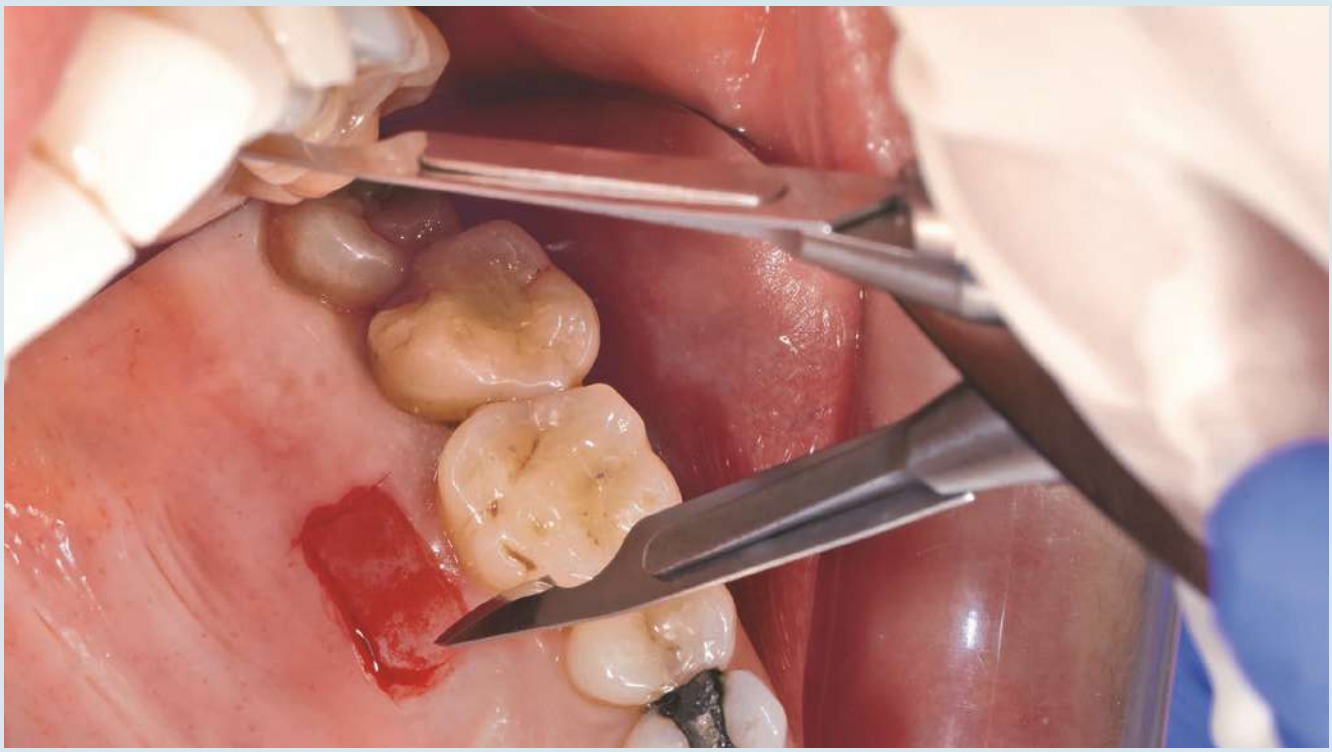


Introduction

The use of a connective tissue graft (CTG) for the purpose of increasing the width of keratinized gingiva was first described by Alan Edel in 1974.[1] Since then, many improvements have been suggested to meet the needs of the continually evolving fields of periodontal plastic surgery and implantology. Besides widening of the keratinized tissue, increasing the soft tissue volume is the other main goal of soft tissue grafting interventions. As a consequence of the higher esthetic and functional demand, soft tissue replacement grafts are widely used to treat a variety of procedures such as soft tissue augmentation, ridge preservation, recession coverage, papilla reconstruction, furcation treatment as well as the management of tissue abnormalities such as scar corrections.[2-5]

Three histologic layers can be fractioned in the masticatory mucosa of the hard palate, which served as a donor site in the present study: the epithelium, the lamina propria, and the submucosal layer. The epithelial layer is about 300-µm thick and is characterized by orthokeratinization. Only a few articles in the literature define the epithelial thickness for palatal mucosa, ranging from a mean of 364 µm to 430 µm.[6,7] Additionally, an association was found for epithelial thickness and the following characteristics: age, gender, donor site, smoking, and the presence of removable devices.[6-10]

The aforementioned deeper layers of lamina propria and submucosa compose the connective tissue. The lamina propria consists predominantly of collagen fibers and is divided into the papillary and reticular portions. The papillary portion is the most superficial part, with finger-like projections that interlock with the overlying epithelium. Thick and dense reticular fibers embody the reticular portion.[11,12]

Whereas the lamina propria is composed of coarse tissue, the submucosa comprises mainly adipose tissue with numerous glands and nerves. This more profound layer has a great variability in composition and dimension, inter- and intraindividually.[4,13]

Despite the widespread usage, high esthetics, and predictable results of CTG procedures,[4] previous studies have demonstrated multiple complications, including graft necrosis,[1] sloughing of palatal tissue,[14] paresthesia or hemorrhage,[12] cyst formation,[15,16] swelling,[17] and gingival cul-de-sac formation.[18] Residual epithelium is assumed to be responsible for some of these postoperative complications, as histologic analyses of biopsies from cysts, swellings, and gingival cul-de-sacs found the presence of epithelial cells.[15-18]

Nowadays, a variety of different harvesting techniques with numerous modifications is available with the main goal of obtaining the largest possible volume while minimizing postoperative discomfort such as pain, bleeding, and morbidity. Two groups can be distinguished: subepithelial connective tissue grafts (SCTGs) and deepithelialized gingival grafts (DGGs), or CTGs with and without a remaining collar of keratinized epithelial lining.[19]

Depending on the desired geometric shape and histologic composition of the autografts, a remarkable number of clinical suggestions is proposed for both harvesting technique and grafting site. Nevertheless, to date, the clinical decision depends on the personal preference of the treating surgeon.[4]

The aim of the present preliminary single-center study was to observe and make a histologic comparison of a CTG harvested from the lateral palatal mucosa through the use of two different harvesting methods: One graft was retrieved from its lining epithelium intraorally by a coarse diamond bur (T), and the other was deepithelialized extraorally with the aid of a no. 15c blade (C). The main hypothesis of the present study proposed a

more consistent result for the more conventional deepithelialization by blade.

Materials and methods

Patients

Three patients, two females and one male, were enrolled in the present study, which took place at a private referral practice for periodontology in Bulgaria, with procedures requiring CTGs for different purposes. The average patient age was 42.3 (30 to 62) years. All patients were Caucasians and were in good general health. Patients with mucocutaneous disorders, with previous periodontal surgery in the palatal area, on medication affecting the periodontal soft tissue (ie, calcium channel blockers or phenytoin), with uncontrolled systemic diseases as well as anticoagulated patients were excluded from the study. None of the patients showed signs of inflammation, and all patients had < 2 mm of probing depth near the palatal donor site. All patients were informed about the study purpose and the surgical procedure to be used. A consent form agreeing to participate in the study was signed by all three participants (Table 1).

Table 1 Distribution of patient-related factors

	Patient 1	Patient 2	Patient 3
Age (years)	62	35	30
Gender (male/female)	f	f	m
Ethnicity	Caucasian	Caucasian	Caucasian
Smoking status (Y/N)	No	Yes	No

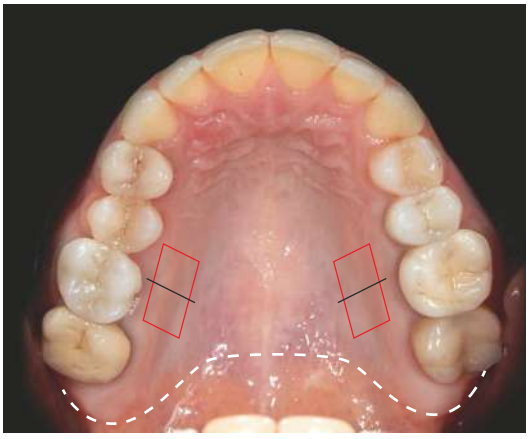


Fig 1 Setup: Two equally sized grafts (red outlines) were harvested using two different deepithelialization techniques. After bilateral deepithelialization of both sides, each of the six grafts was cut vertically into two equally sized pieces. A small biopsy adjacent to this middle-section cut (black line) was processed for further histologic observation.

Graft harvesting

Each of the three study participants provided two equally sized grafts from both sides of the lateral palate, located 2 mm from the gingival border of the maxillary first molar. After delivering local anesthesia of 4% articaine with 1:100,000 epinephrine, two sets of two parallel incisions with a depth of 2 mm perpendicular to the masticatory mucosa were set to outline the graft. All incisions were performed with a no. 15c blade.

All grafts were equal in size with a dimension of 6 mm (vertically) by 10 mm (horizontally) (Fig 1). The grafts were obtained by one well-experienced periodontist under magnified vision using an operative microscope (Zeiss ST; Carl Zeiss).

Two different methods, each on one side of the palate, were deployed to harvest the replacement grafts.

With the first rather novel method, the lining epithelium of the palatal graft was deepithelialized intraorally before harvesting with a round coarse diamond bur mounted on a low-speed contra-angle handpiece (Fig 2a). Graft retrieval was fulfilled by rotating the blade almost parallel to the mucosal

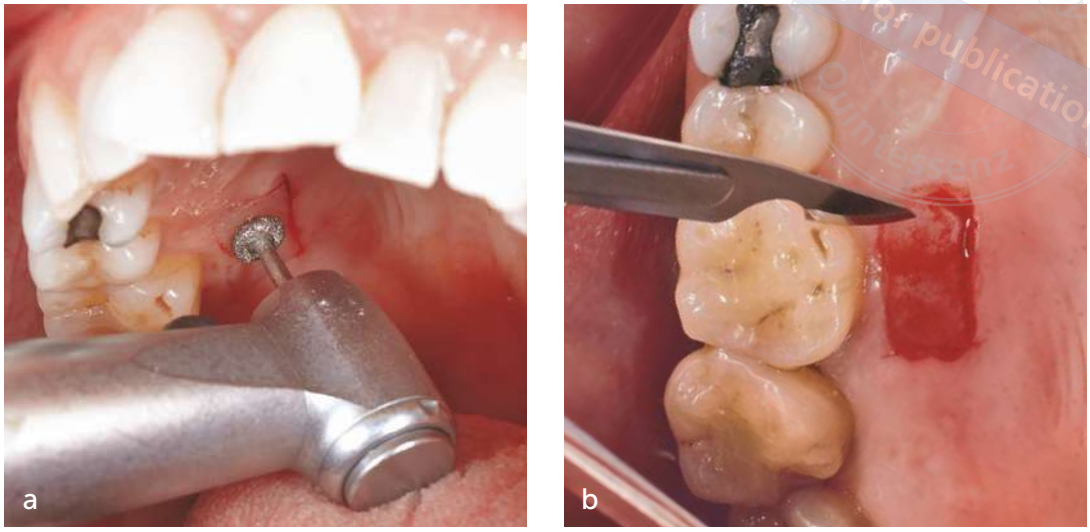


Fig 2 Method one (T): (a) The lining epithelium of the palatal graft was deepithelialized intraorally before harvesting with a round coarse diamond bur mounted on a low-speed contra-angle handpiece. (b) The upcoming bleeding allowed control of the depth of deepithelialization. The excised partial-thickness graft had a remaining thickness of 1.5 mm.

surface and moving apically until mobilization. The upcoming bleeding allowed the practitioner to control the depth of deepithelialization. The excised partial-thickness graft had a remaining thickness of 1.5 mm (Fig 2b).

The second method can be described as more conventional. After preparing the outline of the graft, a free epithelium CTG was harvested with a no. 15c blade from the lateral aspect of the palate with a uniform thickness of 2 mm (Fig 3a). The obtained graft was positioned on a wooden spatula, moistened with a saline solution, and excised of its lining epithelium with a fresh, sharp no. 15c scalpel blade held parallel to the external graft surface.

After extraoral deepithelialization of the graft, where care was taken to ensure the total removal of the epithelium, a thickness of around 1.5 mm remained (Fig 3b).

All the surgical procedures were performed in one session.

Histologic evaluation

After procuring the grafts from the lateral palatal areas, external pressure was applied at the donor site for 7 min with a wet gauze to obtain hemostasis. After deepithelialization, each of the six grafts was cut vertically, along the short axis of the graft, into two equally sized pieces, and processed for embedding in paraffin. A small biopsy adjacent to this middle-section cut was taken at this time and immediately fixed in 10% neutral formalin solution for further histologic evaluation. Semi-thin (2- to 4-µm) serial sections were cut and stained with hematoxylin and eosin. The sections were observed under a light microscope (Fig 4).

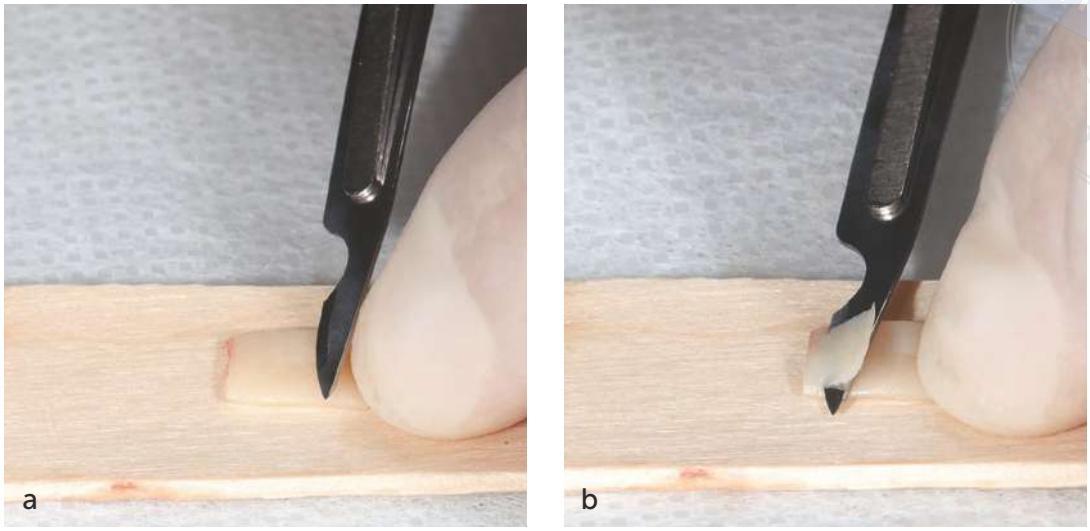


Fig 3 Method two (C): (a) After preparing the outline of the graft, a free epithelium CTG with a thickness of 2 mm was harvested with a no. 15c blade from the lateral aspect of the palate. (b) The obtained graft was positioned on a wooden spatula, moistened with a saline solution, and excised of its lining epithelium with a fresh, sharp no. 15c scalpel blade held parallel to the external graft surface.

Results

The most common overall feature of the six different replacement grafts was the consistency in thickness and proportion. Although the grafts were harvested using two different methods, a general thickness of around 1.5 mm was found for all finalized grafts (Fig 4).

Furthermore, no significant variation in the histologic makeup was found. The six representative grafts were composed predominantly of lamina propria, with the clear appearance of dense collagen fibers surrounded by small blood vessels. No apparent differences in consistency and vascularization were found between the conventional technique and the new deepithelialization method using a bur. Although only a minimal amount, all the grafts harvested with a blade showed a portion of submucosa (Fig 4a), whereas only one out of the three grafts harvested after deepithelialization with a bur revealed some submucosa (Fig 4b-III).

Epithelial remnants, although present in different proportions, were clearly evident in five out of the six grafts. Therefore, only one graft could correctly be considered 'epithelium-free' (Fig 4b-I). Despite the fact that only the most superficial layer of epithelium was excised in all of the extraorally deepithelialized grafts, they all consisted of some residual isolated fragments of rete pegs (Fig 4a). In two out of three grafts where the epithelium was retrieved extraorally, the surface containing residual epithelium was larger than the epithelial-free surface (Fig 4a-II–III).

A remarkable finding was that, in two out of three grafts deepithelialized with a bur, the full elimination of epithelium was

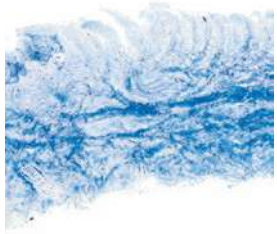
(a) Histologic appearance of CTGs after **extraoral deepithelialization with a conventional blade**. Although only the most superficial layer of epithelium was excised in all of the extraoral deepithelialized grafts, they all contained some residual epithelium.	(b) Histologic appearance of CTGs after a novel **intraoral deepithelialization technique with a coarse round bur.** Although the most predominant part of the graft is composed of lamina propria, all harvested grafts show a portion of submucosa.
 I	 I
 II	 II
 III	 III
 IV	 IV
Highlight of 4a-III: This histologic coupe highlights the presence of almost the entire epithelium layer. Only the most superficial part is excised.	Highlight of 4b-III: This histologic coupe highlights the superficial part of the epithelial excised graft. Although the connective tissue papillae presented intact at this location, a full elimination of epithelium including the rete pegs is displayed.

Fig 4a and b Histologic evaluation under a light microscope.

apparent, including the protruding rete pegs. Nevertheless, the connective tissue papillae displayed an intact presence at that same location (Fig 4b-II–III).

The patients reported a rather minimal level of palatal discomfort pre- and postoperatively, with no need for further unscheduled appointments. Postsurgical healing was uneventful for both grafting sites in all patients.

Both surgical techniques were executed as planned. Nevertheless, the practitioner reported that the deepithelialization with a bur was more technique sensitive due to the thinner and more fragile size and deeper position of the graft that had to be harvested after the intraoral removal of the more fixed-structure epithelial lining. The extraoral deepithelialization by blade was found to be more conventional and therefore easier to perform.

Discussion

A numerous variety of harvesting methods is available nowadays. A distinction can be made between two major CTG groups: SCTGs and DGGs. The former group includes CTGs harvested underneath the epithelial border, with the epithelial layer remaining in situ, while the latter group includes all harvested grafts with a remaining collar of keratinized epithelium. These grafts are excised from their upper layer extraorally.[4,19,20]

The main purpose of the present study was to observe and compare the histologic differences of a CTG harvested from the lateral palatal mucosa using two different harvesting methods. One graft was retrieved from its lining epithelium intraorally with the use of a coarse diamond bur, and the other was deepithelialized extraorally with the aid of a no. 15c blade. The latter control (C) group can be seen as a classic example of a DGG, whereas the former test (T) group is a novel proposed deepithelializing technique that represents a modification of the SCTG.[21]

A limited number of articles in the literature define a mean palatal epithelial thickness

and the possible criteria that have an influence on this value. Lee and coworkers[7] described a mean epithelial thickness of the palatal mucosa of 430 µm, ranging from 113 to 823 µm. While no association was found with donor site or age, significant differences were shown to occur between the genders, with males having much thicker palates than females. These authors suggested to bear in mind a minimum of 0.9 mm for epithelial removal to transcend the greatest epithelial thickness value of 823 µm. It is noteworthy that these higher values may be related to the geographic origin of patients. The diet of the investigated Korean population is divergent, pungent, and spicy, which could influence epithelial thickness.[7,8] Soehren et al[6] reported that the mean epithelial thickness for 14 American patients was 364 µm, ranging from 111 to 619 µm, and besides gender and ethnicity, can also be influenced by donor site, age, smoking, and the presence of removable devices. In this report, a thickness of 0.5 mm was proposed as the excision depth to be borne in mind for the epithelium.[6]

Despite the use of high magnification, microsurgical instruments, and proper lighting under the direct visual inspection of a highly experienced expert in the field, all of the extraoral deepithelialized grafts consisted of epithelium.[4,22,23] With almost all grafts still containing residual epithelium, the chances are that the epithelial thickness was visually underestimated by the well-experienced clinician in the present study. The boundary between the oral epithelium and the connective tissue has to be seen as a high-peak wavy course. The finger-like projections of connective tissue – the connective tissue papillae – project into the epithelial layer. Besides the mean thickness of the epithelial layer, a few studies highlight the age-dependent differences in composition and the height of the rete pegs,[24,25] whereas others show that the connective tissue papillae of the epithelium–connective tissue interface consist predominantly of loose connections in older patients.[7]

In the present study, an assumption can be made that the peaks of the connective tissue papillae were already visible, although the rete pegs were still in situ. The visual confirmation of connective tissue led to the false assumption that the deepithelialization process had been completed.

While it can be taken as read that the complete absence of residual epithelium is not a definite criterion for clinical postoperative complications, several studies have shown that epithelial remnants may have an impact.[15-18] Although CTG procedures in general yield excellent clinical outcomes, with rather low postoperative complications, an insufficient exclusion of epithelium could play a negative role in the early postoperative phase, slowing down plasmatic circulation and revascularization, whereas both these factors are considered key to grafting success. Already in 1968, Sullivan and Atkins[26] suggested that fat and glandular tissue should be removed from CTGs due to the fear that they may act as a barrier to these proposed key factors.

Besides the negative influence of 'acting as a barrier,' the epithelial layer itself is less vascular, which will compromise the direct reestablishment of reusing the preexisting vascular network and the subsequent ingrowth of capillaries and anastomoses formation.[27,28] The supposition of switching the superficial epithelial side of the graft inwards or outwards was found to have no significant effect on the clinical outcome.[29]

With other previous studies demonstrating clear evidence of remaining epithelium in up to 80% of grafts without high complication numbers and with high success rates,[30,31] the speculation can be made that there is a sort of threshold for the portion of epithelium impacted, without leading to further complications.[4]

In the present study, the histologic coupes obtained by the novel intraoral deepithelialization technique (T) showed a remarkable finding: Despite the fact that the connective tissue papillae presented intact at some locations, a full elimination of epithelium including the rete pegs was displayed.

A basal lamina of around 350 nm thickness joins the epithelium to the underlying connective tissue. Anchoring fibrils physically connect the hemidesmosomes of the basal epithelial cells to a reticular condensation of the underlying tissue fibrils. There they appear to form loops around interstitial collagen fibers.[11,12] Apparently, the coarse sweeping movement detaches the epithelium–connective tissue entanglement without causing damage. The brushing movement of the low-speed bur seems to disconnect the anchoring fibers of the basal lamina.[32,33]

Considering the growing demand for excellent esthetics and function, an increased usage and popularity of CTGs can be expected. Therefore, a general basic understanding of mucosal anatomy and the fundamental biologic principles of CTG integration and vascularization are essential for success in clinical applications.

Conclusions

Within the limitations of the small number of clinical cases included in the present study, the presented in situ deepithelialization technique for CTG harvesting using a bur, which is assumed to require higher practitioner expertise to execute, seems to be more predictable in outcome than extraoral epithelial excision by blade. Contrary to expectation, a greater portion of epithelial remnants was found for this latter, more conventional method. Future research in periodontal plastic surgery, including further long-term clinical and histologic studies with a greater number of samples, should focus on comparing graft deepithelialization of a CTG using a coarse bur with that using a standard blade.

Disclaimer

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